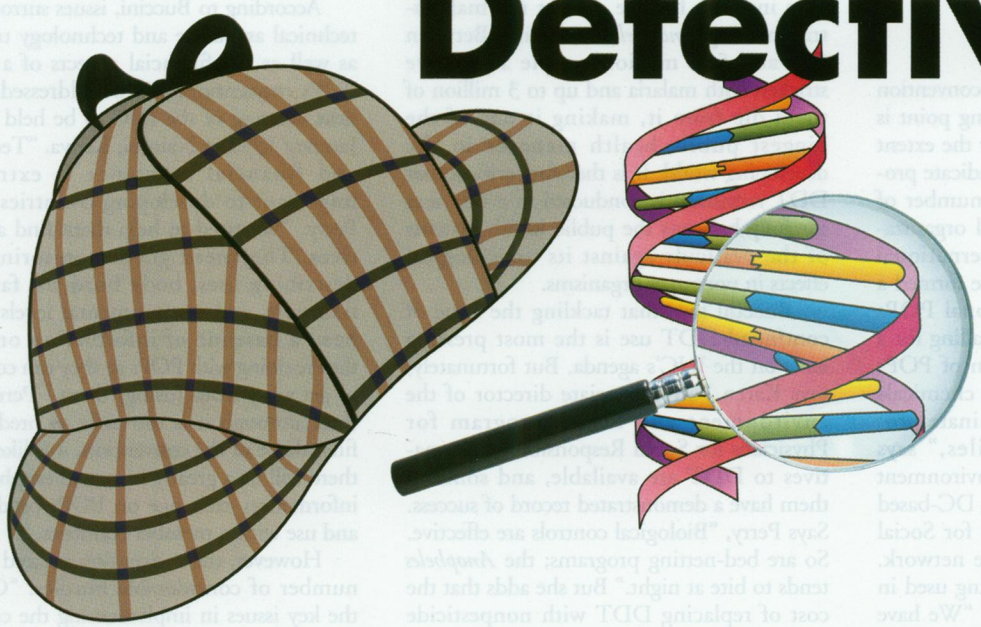
DNA Detectives



Damage to chromosomes, or DNA, plays a fundamental role in both aging and developing cancer. When chromosomes become damaged, it can lead to cell mutations or DNA that cannot be replicated, both of which can be lethal to a cell. Most people know their DNA can be damaged by nuclear radiation, cigarette smoke, or the sun's rays, but simply living damages DNA, too. Susan S. Wallace, a molecular biologist at the University of Vermont in Burlington, says, "It's been 'estimated'—and I have to emphasize estimated—that there's somewhere between 10,000 and 20,000 oxidative damages produced per cell per day, just because we breath air. Our genes get damaged as a by-product of metabolism." To understand the potential causes and resulting consequences of DNA damage, scientists need methods to detect it. X. Chris Le, an analytical chemist at the University of Alberta in Edmonton, Canada, and colleagues recently developed an extremely sensitive method for measuring such damage.

DNA can be damaged in many ways. For example, a strand can be broken or one of its bases—adenine, cytosine, guanine, or thymine—can be chemically modified. In most cases, biochemical systems repair the damage, and when a healthy cell's DNA is damaged, repair is beneficial. On the other hand, repair also serves as an obstacle to some forms of cancer treatment, such as when irradiation is used in an attempt to kill tumor cells through damaging their DNA. In either case, to know how much DNA is being repaired, scientists must first know how much is being damaged.

Le recalls that a few years ago he began studying DNA damage and found that the techniques for measuring it seemed insufficient for detecting the DNA damage occurring due to low-dose environmental or clinical exposures. "So," he says, "I decided to try to develop a new approach." Le teamed up with Michael Weinfeld, a biochemist at the Cross Cancer Institute, also in Edmonton. Together, they began developing a selective, sensitive technique for measuring DNA damage.

Looking for Labels

First, the scientists needed a way to label damaged DNA. For that, Weinfeld turned to Steven Leadon, a molecular biologist at the University of North Carolina at Chapel Hill, who had developed an antibody to thymine glycol. Formation of thymine glycol is one of the predominant types of base modifications caused by ionizing radiation, and it's formed at exposure to a relatively high level of radiation. Says Leadon, "Thymine glycol blocks replication, so it has the potential of being a lethal lesion in the cells. It's also a block to transcription, and that could alter the types of RNA and subsequent proteins that were being made." His antibody finds a single thymine glycol and attaches to it.

Using this antibody, Le, Weinfeld, and Leadon could selectively tag one type of DNA damage, but they needed a way to locate the tag. This can be a challenge, Le says, because only a very small portion of DNA is damaged to form thymine glycol. The researchers needed a sensitive technique to detect the damaged portion. They chose laser-induced fluorescence, one of the most sensitive techniques available. To use this technique, however, the combination of the antibody and thymine glycol would need to fluoresce under laser light, which it doesn't normally do. So Le and colleagues added a second antibody—one connected to a fluorescent molecule—that would attach to the thymine glycol antibody.

In the detection process, DNA is extracted from cells and mixed with both the primary thymine glycol antibody and

the secondary antibody made with the fluorescent molecule. In the mixture, fluorescence can come from three sources: a free secondary antibody, a complex of secondary and primary antibody, or a combination of secondary antibody, primary antibody, and thymine glycol. Measuring the last combination provides the information on DNA damage.

To differentiate between the fluorescence combinations, the team uses capillary electrophoresis, a high-resolution separation technique. The DNA–antibody mixture goes into a tube about the size of a human hair, with an internal diameter of 10–100 µm. Next, a high voltage source placed across the ends of the tube separates the various components in the mixture on the basis of charge, mobility, and size. As the fluorescent components exit the tube, a laser induces fluorescence, which can be quantified. Control experiments can be conducted to distinguish between the various fluorescing components. The higher the fluorescence of the secondary antibody/primary antibody/thymine glycol component, the higher the DNA damage.

Totaling the Advantages

In experiments published in the 15 May 1998 issue of *Science*, Le and his colleagues reported that their technique "represents an improvement of 4 to 5 orders of magnitude over currently available assays for DNA base damage." Such sensitivity allows this technique to measure the DNA damage resulting from clinical levels of radiation, which is generally a dose of 2 Gray (Gy) or less. For example, Le and his colleagues irradiated A549 cells, which come from a human lung carcinoma line, and were able to detect levels of thymine glycol after doses as small as 0.05 Gy. In fact, the technique found thymine glycol at levels of 1 damaged base in 1 billion normal bases.

This level of sensitivity could reveal the day-to-day levels of DNA damage. "You do find a steady-state level of damage, but the estimates of those steady-state levels are still fuzzy," says Wallace. "That's why this particular technique will be very useful, because it will enable us to detect very low levels of damage—the steady-state levels that our cells are sustaining at any one time. The benefit would be that we would have a baseline, and from an environmental perspective, when you're trying to measure what effects environmental or exogenous toxicants have on the cell, you would know the basic level of damage the cell has and is both tolerating and capable of repairing on a daily basis."

Beyond sensitivity, this new technique offers other advantages. First, it does not require digesting, or breaking up, the DNA, in contrast to many other approaches. Using intact DNA should lead to more accurate measurements of DNA damage, because the digesting processes themselves generate damage that distorts findings. Perhaps most important, this technique is expected be adaptable to all sorts of DNA damage. By changing the antibody or using another form of recognition protein, this technique could be used to detect many kinds of damage. For instance, Wallace is experimenting with using antibodies to various forms of DNA damage, including thymine glycol, 8-oxoguanine, 5-hydroxylcytosine, and uracil glycol, in Le's system. Says Wallace, "We're not at the state yet where we actually can say we have really good data, but we're working on it."

This technique should also be economical for several reasons. It uses much smaller amounts of biochemicals such as antibodies, and much smaller amounts of DNA (nanograms, compared to micrograms) than traditional detection methods, so the cost of the materials is less. The equipment itself is not very expensive, either. Says Le, "We constructed our own equipment for about $30,000 or so, but there are also commercial instruments available." And in the long run, says Le, the technique will be cheaper because it's not as labor-intensive as other assays. "Once it becomes routine," Le says, "I wouldn't think this [would be] more expensive than other techniques."

The most significant disadvantage of the technique is the level of expertise required. "For now, it requires a lot of expertise to make the assay successful," Le says. "With good analytical expertise like [that of our people], they can have it working. But as a commercial, routine analysis tool, perhaps it [will] take a while for people to get used to the specific details." Le thinks the trickiest part of the process involves the capillary electrophoresis, which is less commonly used than, say, high-performance liquid chromatography.

Areas for Application

Le and his colleagues, as well as other experts in the field, expect this technique to open the way to many new applications of measuring DNA damage. For instance, it could assess the actual risk of exposure to a toxic substance by measuring the resulting DNA damage. "One of the difficulties people have in trying to assess risk is that you often end up giving animals very high doses of an agent in order to see an end point, and then you hope that it's a straight line back to zero," says Leadon. "What we're hopefully being able to do is to fill in that gap between someone who is totally unexposed and . . . somebody who has received a high dose, and be able to see what are the real risks involved."

Bruce N. Ames, a molecular biologist at the University of California at Berkeley, says, "I think this [method] is a very important technical advance. . . . What it shows is that there's a huge background of DNA damage going on. But it will help to put things in perspective. . . . I think the important use of these new techniques is to say, 'What are the big risks out there?'"

Oncologists could use the technique to study various cancer treatments. For example, in their *Science* article, the authors examined a potential problem with radiation treatments, which are generally given in a series of small doses. The first doses can induce cells to resist the effects of later doses. To study this phenomenon, Le's team irradiated A549 cells with either 2 Gy of radiation or 0.25 Gy followed 4 hours later by 2 Gy, and then measured the removal of thymine glycol—a measure of how well the cancerous cells repaired the radiation-induced DNA damage. The cells that received the small priming dose repaired their DNA much faster, reducing the time for 50% removal from approximately 100 minutes to approximately 50 minutes. By knowing how the cancerous cells fight against this treatment and others, scientists might create more effective treatment approaches.

Mike May

Suggested Reading

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Le CX, Xing JZ, Lee J, Leadon SA, Weinfeld M. Inducible repair of thymine glycol detected by an ultrasensitive assay for DNA damage. Science 280:1066–1069 (1998).

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